

CD4+ γ/δ T-cell leukemia/lymphoma: clinical, immunophenotypic and molecular aspects in an unusual case

Haematologica 2007; 88;(9)e120-e122

T-cell lymphomas represent a problematic diagnostic group, most cases arising from $\alpha\beta$ T-cells. Malignant $\gamma\delta$ T-cell lymphoproliferations are uncommon and need more precise clinical and biological characterization.¹ We describe here clinical, immunophenotypic and molecular analyses of a particular case of CD4+ $\gamma\delta$ T-cell leukemia/lymphoma. A 74-year old white man was hospitalized with a twenty-day history of generalized pruritus, profuse jaundice and nocturnal fever (38°C). Physical examination showed an enlarged liver and spleen (9 cm and 4 cm b.c.m., respectively) without lymph node enlargement. The hemogram revealed hemoglobin 12.2 g/dL, platelet count $72 \times 10^9/L$ and leukocyte count 79.3×10^9 cells/L with 87% lymphocytes. Liver function revealed bilirubin levels of 10.46 mg % and LDH 1370 U. Serological tests for HCV, CMV and HTLV-1 were negative. Bone marrow (BM) biopsy showed diffuse interstitial infiltration of CD45RO+ and CD3+ cells (Figure 1.). Peripheral involvement was confirmed by CD99 negativity (DAKO). BM and peripheral blood (PB) smears showed medium sized cells with moderately condensed chromatin, nucleoli and absence of detectable cytoplasmic granules. Abundant mitotic cells were observed in PB smears (Figure 2.). Initial treatment was based on chlorambucil (Leukeran®) and prednisone with no response. Thereafter a COP scheme was instituted. The patient deteriorated progressively with an increase in leukocyte count up to 120×10^9 cells/L in the third week and died after 20 days of hospitalization. Hemophagocytic features and disseminated intravascular coagulation were not observed during the course of the disease. His family did not authorize post-mortem analyses.

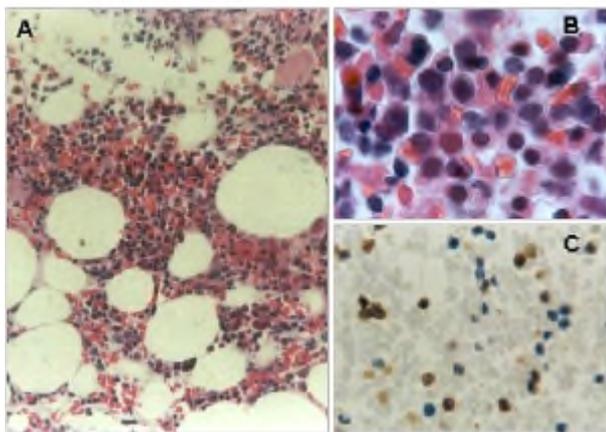


Figure 1. Bone marrow histopathologic analyses. (A) Diffuse interstitial infiltration (H&E; original magnification X100). (B) A high magnification of 1A showing an infiltrating abnormal population of lymphoid cells (H&E; X1000). (C) Antibodies specific for CD3 (UCHT1, 1:200, DAKO) manifest diffuse positivity (Immunoperoxidase stain, original magnification X 400).

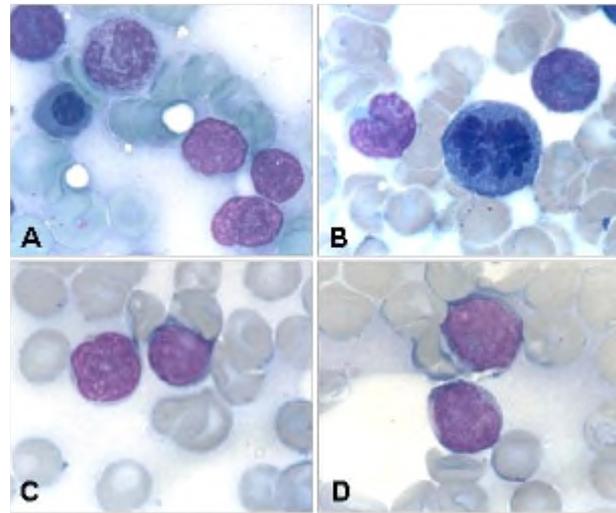


Figure 2: Bone marrow (A) and peripheral blood (C-D) smears. Note irregular nuclei and presence of nucleoli in small- to medium-sized abnormal lymphocytes. In (C) a mitotic cell is indicated by an arrow. (May-Grünwald Giemsa stain, X1000).

Flow cytometry using monoclonal antibodies against a panel of B and T-cells (Becton Dickinson) showed a homogeneous T-cell population with expression of CD7 (99.7%), CD2 (97.63%), CD5 (99.7%), CD3 (99.49), CD4 (55.5%) and the $\gamma\delta$ T-cell receptor (TCR) (55.5%). $\alpha\beta$ -TCR, CD1a, CD8 and B-cell antigen expression were negative. EBV and HHV-8 genomes were not detected by PCR in tumor cells. TCR γ and TCR δ clonalities were assessed by PCR with primers for the most used V γ , V δ and J γ and D segments, followed by heteroduplex analysis.^{2,3} The presence of a complete V δ 1(DD)J δ 1 clonal rearrangement (Figure 3A) and V γ I-J γ 1.3/2.3 and V γ 9-J γ 1.3/2.3 clonal rearrangements (Figure 3B.), was determined. $\gamma\delta$ T-cells comprise about 4% of T lymphocytes. In peripheral blood of healthy adults, 70-90% express a V δ 2/V γ 9 TCR, while 10-30%, express V δ 1 with any V γ .⁴ The latter is the predominant resident population in normal spleen and liver. $\gamma\delta$ T-cell lymphomas include a group of aggressive diseases whose differential diagnosis is complex.^{1,5} Besides hepatosplenic $\gamma\delta$ T-cell lymphomas (HSTL) which display a quite distinct morphologic, immunologic and clinical picture,⁶ $\gamma\delta$ T-cell lymphomas can arise at different extranodal non-hepatosplenic locations (skin or mucosae)⁷ and shows substantial morphological heterogeneity. In general terms, it could be considered that HSTL arise from V δ 1+ $\gamma\delta$ cells while non-hepatosplenic lymphomas arise from V δ 2+ $\gamma\delta$ cells. Immunomolecular characteristics of the reported case indicated a V δ 1 $\gamma\delta$ T-cell population expressing CD4+ as a target for leukemic transformation. It may represent a HSTL with early leukemic spread or an aggressive variant of $\gamma\delta$ large granular leukemia as described by Gentile *et al.*⁸ Lack of spleen/liver microscopic examination prevented detection of only intrasinusoidal/sinusual infiltration, typical of HSTL. BM interstitial infiltration is not

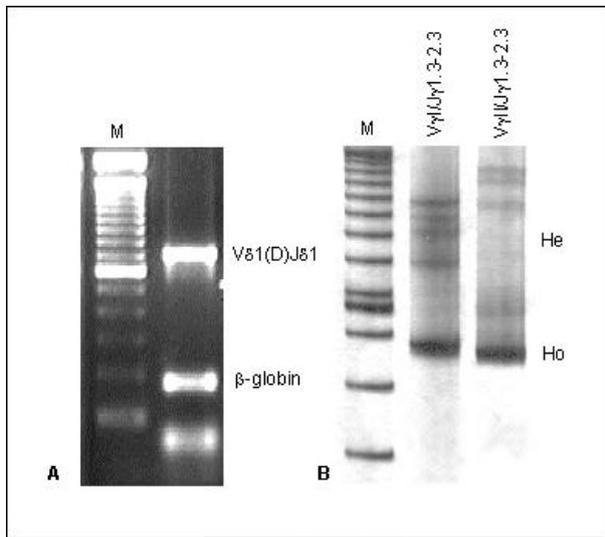


Figure 3: PCR analyses of TCR γ and g gene rearrangements. **A:** TCR γ rearrangement V δ 1(DD)/J δ 1 (750 bp). A 167 bp fragment of b-globin constitutive gene was used as an internal control. Agarose gel (2%), ethidium bromide. **B:** TCR α rearrangements followed by heteroduplex analysis: V α I/J α :1.3-2.3 and V α II/J α :1.3-2.3 rearrangements. Ho: Homoduplex; He: Heteroduplex. M: Molecular weight marker (100 bp ladder). Native polyacrylamide gel (8%) with silver staining.

uncommon, and leukemic transformation is a frequent final manifestation of HSTL. However, the observed cell morphology, the expression of CD5 (very infrequent) and of CD4 antigens (never described in this entity), argue against this possibility. Some of the morphologic and immunophenotypic features observed in the leukemia herein described resemble T-cell prolymphocytic leukemia (T-PLL), for instance the expression of a complete pan-T profile, including CD4 marker in 55% of cells. Sugimoto *et al.*⁹ described a case of $\gamma\delta$ T-PLL in a 30-year old man, but that leukemia showed a CD4-, CD8-phenotype. The present case has several unusual aspects. First, its course was fulminant; there are few cases described with that outcome despite aggressiveness being characteristic of $\gamma\delta$ T-cell lymphoproliferations.^{5,6} Second, these diseases are more frequent in young patients, with few cases occurring over fifty years.^{5,6} To our knowledge, this is the oldest reported patient having a $\gamma\delta$ T-cell lymphoma/leukemia. V δ 1 T-cell populations might be *at risk* of leukemic transformation in elderly patients as a result of processes of immunosenescence¹⁰ as suggested by the findings that the V δ 2/V δ 1 ratio is inverted in old subjects and that V δ 1 T cells are more activated^{10,11} pointing to a role of the latter in replacing the immune deficit in the elderly. This agrees with the suggestion that impaired immune function may have a pathogenic role in $\gamma\delta$ T-cell malignancies such as HSTL.¹² Third, there was an important leukocytosis at the expense of CD4+ T cells with normal hemoglobin levels and non-severe thrombocytopenia. $\alpha\beta$ and $\gamma\delta$ T-cells are characterised by the expression of a similar subset of pan T cell markers. Most $\gamma\delta$ T cells -and also

most $\gamma\delta$ T cell lymphomas- have a CD4-CD8-immunophenotype. The rare CD4+ $\gamma\delta$ T cell clones produce large amounts of cytokines^{4,5} which may have had a differential effect on the haematological profile of the disease here reported. Here we describe the oldest known patient with a $\gamma\delta$ T-cell malignancy, with a very acute onset and a pan-T-CD4+ immunophenotype. The scarcity of similar cases makes delineation of a clinical-pathological entity difficult. Instead, it would be important to consider the functional redundancy of the immune system in the origin of different and perhaps equivalent $\alpha\beta$ and $\gamma\delta$ neoplasias. Inclusion of $\gamma\delta$ lymphoproliferations in diagnostic schemes is also very important because without complete immunophenotyping and molecular confirmation of monoclonality, the histopathologic findings can be difficult to interpret.

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Acknowledgments: We thank Dr. Sergio Romano (Pathology Section, INCA, Rio de Janeiro, Brazil) for the immunochemistry analysis and Dr. Claudio Bidau for critical review of the manuscript. This work was supported by PRO-VITA Association, FAPERJ and INCA.

Key Words: $\gamma\delta$ T-cell leukemia/lymphoma, immunophenotype, $\gamma\delta$ TCR.

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